Changes of the Gingival Blood Flow in Rats with Congenital Susceptibility to Gingivitis

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ABSTRACT. The gingival blood flow was measured by hydrogen gas clearance method in rats with naturally occurring gingivitis (SUS rat). A significant increase in the blood flow was observed in the gingiva of 2- to 4-month-old SUS rats with acute gingivitis. Thereafter, the gingival blood flow gradually decreased. The blood flow in the gingiva with subacute gingivitis showed almost similar to that in the gingiva of SUS rats at pre-experimental stage. The control rats (RES rats) showed constant gingival blood flow during the experimental period. The findings suggest that changes in the gingival blood flow are associated with the process of gingivitis.—**KEY WORDS:** blood flow, gingiva, gingivitis, SUS rat

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Periodontal disease is an inflammatory and destructive lesion in the gingiva caused by plaque bacteria [9–11]. The inflamed gingival tissues show various histological features with the progression of the disease [16, 21]. Acute gingivitis is evidenced by severe edema and neutrophilic infiltration, often with necrosis of the gingival epithelium. The lesion progresses to the inflammation rich in plasma cells and lymphocytes, sometimes with slight fibrosis [21].

In general, changes in local blood flow reflect the condition of the local environment. For instance, decreased blood flow results in a lack of oxygen uptake by gastric tissues [8]. Under this condition, histologically, the tissues involved show degenerative and necrotic changes. Changes of the vascular morphology and permeability in the inflamed gingiva have been shown previously [2, 3, 6, 17, 23, 24]. It is conceivable from these studies that the local blood flow in the inflamed gingiva is reflective with these morphological and physiological changes. However, it has been undefined whether there may be differences in blood flow proceeding and various phases of

periodontal disease. There has been insufficient quantification of the degree of changes in blood flow or the severity of periodontal disease. Hock *et al.* [7] have reported that no difference of blood flow is found between uninflamed and inflamed gingiva. In contrast, some workers have reported that the significant elevation of blood flow is found in inflamed gingiva [14]. In the present study, local blood flow in the gingiva was measured and compared with the progression of gingival lesions using the rats with naturally occurring gingivitis. It seems to provide valuable data for assaying the condition of the gingiva.

MATERIALS AND METHODS

Animals: Rats with naturally occurring gingivitis (plaque susceptible rats: SUS rats) and control rats (plaque resistant rats: RES rats) were used [11, 12]. All rats fed a commercial powder diet from 1 month of age. Numbers of rats examined were 7, 3, 4 and 6 SUS and 3, 3, 4 and 3 RES rats at 1, 2, 4 and 7 months of age, respectively.

Measurement of blood flow: Both SUS

Fig. 1. Acute gingivitis in SUS rat at 2 months of age. Infiltration of many neutrophils and edema are shown.

and 3 RES rats at 1, 2, 4 and 7 months of age, respectively.

Measurement of blood flow: Both SUS and RES rats at 1, 2, 4 and 7 months of age were measured local blood flow in the gingiva. They were anesthetized with Nembutal (20 mg/kg). Blood flow was measured by the hydrogen (H_2) gas clearance method [1]. Local blood flow was determined by measuring the half reduction time ($T_{\frac{1}{2}}$, in min) of the local H_2 gas concentration which was saturated beforehand by respiration. Local H_2 gas concentration was measured by using the 0.3 mm diameter and epoxy-coated platinum microelectrode of which 1 mm in length from the tip was uncoated as a sensor (UHE-100 standard needle type, Unique Medical Co., Japan) connected to UH meter (Unique Medical Co., Japan). Briefly, one of the microelectrodes was inserted into the gingiva of lower incisor. The insertion point of microelectrode was

1.5–3.0 mm distance from the mid-line between the right and left lower incisors, and 2.0–4.0 mm distance from the tip of the gingiva. The other microelectrode was attached to the right leg. Rats breathed H_2 gas within the gingival concentration of H_2 gas indicating saturation level. After H_2 gas concentration in the gingiva reached to the saturation level, supply of H_2 gas was stopped, and $T_{\frac{1}{2}}$ of H_2 gas concentration in the gingiva was measured. The local blood flow value was calculated as follows.

Local blood flow (ml/min/100g) = $\ln 2 / T_{\frac{1}{2}}$

$\times 100 = 69.3 / T_{\frac{1}{2}}$ ($T_{\frac{1}{2}}$ = half reduction time).

Details of this analysis were described by Auckland *et al.* [1] and Kety and Schmidt [15]. The data were analysed by student's *t*-test.

Histological examinations: After blood flow measurement, all rats were sacrificed, from which their gingiva of lower incisors was removed. The tissues were fixed in 10% formalin and decalcified by nitrate acid solution. Then the tissues were embedded in paraffin. Five μ m sections were stained with hematoxylin and eosin for microscopic examinations. The lesions in the gingiva were classified on the basis of the previous description by Isogai *et al.* [11].

Evaluation of plaque accumulation: The amount of plaque accumulation was evaluated by plaque index system [12]. The criteria for this system were as follows: 0: No plaque accumulation, 1: A scarcely detectable accumulation, thinly covering 25% of the gingiva, 2: Plaque covering 50 to 100% of the gingiva, 3: A thick accumulation covering 100% of the gingiva, 4: The thickest and widest accumulation.

Measurement of blood pressures: Blood pressures in the carotid artery were mea-

Fig. 2. Edematous swelling of the gingival blood vessel wall in SUS rat at 2 months of age.

Fig. 4. Change in the plaque accumulation in SUS and RES rats. Amounts of plaque were evaluated by plaque index system. Data in each point are expressed as the mean value of animals examined. Standard deviation is shown by bar.

sured as a representative parameter of the systemic hemodynamic condition using sphygmomanometer.

RESULTS

The gingival lesions including inflammation and plaque accumulation were found in SUS rats after 2 months of age. Histologically, two types of inflammatory lesions, acute and subacute gingivitis, were observed in the gingiva of SUS rats. Acute gingivitis was characterized by cellular infiltration rich in

Fig. 3. Subacute gingivitis in SUS rat at 7 months of age. This lesion progressed from the acute gingivitis shown in Fig. 1 to the plasma cellular proliferation shown in this figure.

neutrophils with edema (Fig. 1). Edematous swelling of the vascular walls was observed in the gingiva of SUS rats with acute gingivitis (Fig. 2). This type of gingivitis was found in all 3 SUS rats at 2 months of age and 3 of 4 SUS rats at 4 months of age examined. Thereafter, the lesions progressed to subacute gingivitis which was characterized by cellular infiltration rich in plasma cells with some neutrophils (Fig. 3). Sometimes, slight fibrotic change was found in the gingival vascular walls. Subacute gingivitis was found in 1 of 4 SUS rats at 4 months of age and all 6 rats at 7 months of age examined. SUS rats at 1 month of age and all RES rats examined had no histological lesions.

Plaque accumulation was found in 2-, 4- and 7-month-old SUS rats but not in RES rats and 1-month-old SUS rats. Changes of plaque accumulation evaluated by the pla-

Table 1. Comparison of the blood flow among gingival inflammatory conditions

Strain of rat	Gingival condition		
	Pre-experimental	Acute inflamed	Subacute inflamed
SUS ^{a)}	86±3(7) ^{c)}	137±10(6) ^{d)}	75±16(7) ^{e)}
RES ^{b)}	65±11(13) ^{f)}	* ^{g)}	*

a) Plaque susceptible rat.

b) Plaque resistant rat.

c) Mean blood flow±S.D. ml/min/100g (number of rat examined).

d) Three rats at 2 months of age plus 3 rats at 4 months of age with acute gingivitis.

e) Six rats at 7 months of age plus 1 rat at 4 months of age with subacute gingivitis.

f) The blood flow of RES rats is the mean of all RES rats examined.

g) None of RES rats showed gingivitis.

que index system are shown in Fig. 4.

Table 1 shows the comparison of the blood flow among rats with acute or subacute gingivitis and with the healthy gingiva. The blood flow at pre-experimental stage was obtained from 1-month-old SUS rats in which any inflammatory change was not observed in the gingiva. The blood flow in RES rats of 1, 2, 4, and 7 months of age was 74±7, 56±2, 63±5 and 67±3 (ml/min/100g), respectively, and the value shown in Table 1 is the mean of 13 RES rats from all age groups examined. No statistically significant difference was observed among these blood flows. SUS rats with acute gingivitis showed high gingival blood flow. A significant difference was found in the blood flow between no inflamed (pre-experimental stage) and acute inflamed gingiva ($P<0.01$). Then, the blood flow decreased. The blood flow in SUS rats with subacute gingivitis was significantly lower than that in rats with acute gingivitis. The blood flow in the gingiva with subacute gingivitis appeared to be lower than that in the pre-experimental gingiva although the difference was not statistically significant.

The systemic hemodynamic condition of animals was estimated by the blood pressures in the carotid artery. SUS and RES rats showed the same blood pressure, 106–109 mmHg.

DISCUSSION

The present study clearly shows the association of changes in the local blood flow with the process of local inflammation in the gingiva. The result indicates that measurement of local blood flow can appreciate the gingival inflammatory conditions and further suggests that difference of the blood flow among the phases of inflammation is one of the causes of the disagreement of the previous studies.

An increase in the local blood flow was significantly correlated with local acute inflammation of the gingiva of SUS rats. The edematous change of vascular walls observed in the gingiva with acute inflammation is recognized as one of the consequences of the increase in local blood flow. This interpretation is supported by the finding that the blood flow decreased with the reduction of the severity of the edema.

An increase in the local blood flow was concurrently observed with the beginning of the plaque accumulation in the gingiva. It has been considered that periodontal inflammation of SUS rats is induced by bacteria in plaque [11, 12]. Plaque has been known to contain many substances showing various biological activities [5, 18–20]. Many studies have indicated similar results [5, 11, 18–20, 22]. Some of the bacterial components such as lipopolysaccharide have been considered as the effective agents to induce a local inflammation and increase in the gingival blood flow. The high blood flow observed in the gingiva with acute gingivitis may be the result of primary exposure to physiological effects of bacterial plaque. Low level of blood flow in RES rats and

pre-experimental stage of SUS rat may be due to the absence of plaque accumulation.

The influences of local inflammation extend to not only blood flow but also permeability in the gingival blood vessels. Actually, a vascular permeability has been found to increase in inflamed tissues [2, 3, 24]. Also in the present study, increase in the vascular permeability may occur in inflamed gingiva of SUS rats. Gingival edema and cellular infiltration are recognized as the result of increase in vascular permeability. It has been known that some of the bacterial components in plaque have an activity to increase the vascular permeability [17, 20]. An increase in the local vascular permeability may be one of the factors to reflect on the change in local blood flow.

The present study also shows the significant reduction of the local blood flow according to the progression of gingivitis from acute to subacute. Similar findings have been reported in the gingiva with chronic gingivitis [4, 7]. These studies have suggested that blood flow rate in the nutritional capillary bed is maintained in chronic gingivitis, but the alteration in blood flow may occur in the non-nutritional blood supply as a result of changes in blood flow through shunts and afferent and efferent vessels. In the present study, fibrotic change of vascular walls was observed in the gingiva of SUS rats with subacute inflammation. The normal or low blood flow value in SUS rats with subacute gingivitis may be correlated with that morphological feature.

The low blood flow has been reported also in the gingiva with atherosclerosis [13]. In such gingiva, the correlation has been evidenced between the low blood flow and the increased numbers of osteoclast which have been recognized as one of the parameters of chronic periodontitis. The report suggests a possibility that the low blood flow in the gingiva with chronic gingivitis may be

not the recovery to normal level. The reduction of blood flow in SUS rats with subacute gingivitis may show similar abnormality because the inflammation was progressive.

The low blood flow in the gingiva with subacute gingivitis could not be explained by correlation with plaque accumulation. A large amount of plaque was observed in the gingiva with subacute gingivitis although the local blood flow showed low value. It seems that some other factors play on the regulation of local blood flow. Further studies will need in this field.

Soft tissue blood flow has been, so far, studied using radio-labeled indicators [4, 7, 14]. It has been pointed out that the method is unsuitable for repeating application to the same animal. In contrast, the H₂ gas clearance technique used in the present study is the simply and highly reproducible method [1]. This method is useful for measuring the blood flow not only in the gingiva but also in various tissues.

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要 約

歯肉炎自然発症ラットの歯肉局所における血流量の変化：磯貝 浩・磯貝恵美子¹⁾・井藤信義¹⁾・高野一雄 (東日本学園大学歯学部口腔解剖学教室, ¹⁾口腔衛生学教室) —— 歯肉炎自然発症ラット (SUS ラット) における歯肉血流量を水素ガスクリアランス法で測定した。急性歯肉炎を起こした2～4ヵ月齢のSUSラットにおいて著明な歯肉血流量の増加が観察された。7ヵ月齢で血流量は対照と同様の値にもどり、歯肉の病変は亜急性炎へと進行した。対照ラット (RES ラット) では歯肉血流量は実験期間中一定であった。この知見から歯肉局所の血流量が急性炎症病変に伴って増加することが示唆された。